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Varying Stable Nitrogen Isotope Ratios of Different Coastal Marsh Plants and Their Relationships with Wastewater Nitrogen and Land Use in New England, USA

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Abstract The stable nitrogen isotope ratios of some biota have been used as indicators of sources of anthropogenic nitrogen. In this study the relationships of the stable nitrogen isotope ratios of marsh plants, *Iva frutescens* (L.), *Phragmites australis* (Cav.) Trin ex Steud, *Spartina patens* (Ait.) Muhl, *Spartina alterniflora* Loisel, *Ulva lactuca* (L.), and *Enteromorpha intestinalis* (L.) with wastewater nitrogen and land development in New England are described. Five of the six plant species (all but *U. lactuca*) showed significant relationships of increasing δ^{15}N values with increasing wastewater nitrogen. There was a significant ($P<0.0001$) downward shift in the δ^{15}N of *S. patens* (6.0±0.48‰) which is mycorrhizal compared with *S. alterniflora* (8.5±0.41‰). The downward shift in δ^{15}N may be caused by the assimilation of fixed nitrogen in the roots of *S. patens*. *P. australis* within sites had wide ranges of δ^{15}N values, evidently influenced by the type of shoreline development or buffer at the upland border. In residential areas, the presence of a vegetated buffer (n=24 locations) significantly ($P<0.001$) reduced the δ^{15}N (mean=7.4±0.43‰) of the *P. australis* compared to stands where there was no buffer (mean= 10.9±1.0‰; n=15). Among the plant species, *I. frutescens* located near the upland border showed the most significant (R^2=0.64; P=0.006) inverse relationship with the percent agricultural land in the watershed. The δ^{15}N of *P. australis* and *I. frustescens* is apparently an indicator of local inputs near the upland border, while the δ^{15}N of *Spartina* relates with the integrated, watershed-sea nitrogen inputs.

Keywords Buffer · Indicator · Macroalgae · Monitoring · Nitrogen loading · *Phragmites australis* · *Spartina alterniflora* · *Spartina patens* · Stable isotopes · Wastewater

1 Introduction

Studies have demonstrated the utility of measuring the stable nitrogen isotope ratios of marsh plants, especially *Spartina alterniflora* Loisel, and using these measures as an indicator of coastal wastewater nitrogen loadings (Cole et al., 2004; McClelland & Valiela, 1998; McClelland, Valiela, & Michener, 1997). *S. alterniflora* is considered a good biological indicator of watershed nitrogen sources in urbanized regions, because the stable nitrogen isotope ratio of *S. alterniflora* reflects the signal of the dominant

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anthropogenic nitrogen sources that are entering the estuary (McClelland & Valiela, 1998; Cole et al., 2004). However, less attention has been given to the use of other salt marsh plants as indicators of sources of anthropogenic nitrogen (Cole et al., 2004). Do differences in marsh plant species and the location of the species on the marsh landscape make a difference when describing relationships between plant stable nitrogen isotope ratios and anthropogenic sources of nitrogen?

High variation in stable nitrogen and carbon isotope ratios for wetland plant species in the San Francisco Bay river-marsh-estuary complex was attributed in part to the location of the plants among estuarine habitats (i.e., salt-marsh creek, freshwater-brackish marsh, river channels, tidal lakes, and riparian corridors) and varying physiology among plant species (Cloern, Canuel, & Harris, 2002). Furthermore, high within species and within plant group variation in stable nitrogen and carbon isotopes among estuarine habitats made it difficult to use stable isotopes as biomarkers of sources of organic matter fueling secondary production in this system (Cloern et al., 2002). However, in this study we are proposing to use stable nitrogen isotope ratios of salt marsh plants as indicators of sources of land-derived anthropogenic nitrogen. Compared with atmospheric deposition (δ^{15}N of +2‰ to +8‰) and commercial, inorganic fertilizers (δ^{15}N of −3‰ to +3‰), nitrogen derived from human wastewater and livestock (δ^{15}N of +10‰ to +22‰) is relatively enriched in N^{15} (Arevena, Evans, & Cherry, 1993; Gormley & Spalding, 1979; Kreitler, Ragone, & Katz, 1978; McClelland et al., 1997). When the percentage of human wastewater that is processed by plants increases, the stable nitrogen isotope ratio in the tissue of the plant is enriched. In contrast, plants that process water with increasing levels of commercial, inorganic fertilizers exhibit a depressed stable nitrogen isotope ratio in their tissues. Plant species such as *Iva frutescens* (L.) and *Phragmites australis* (Cav.) Trin ex Steud are often located in the salt marsh border which may be close to some sources of anthropogenic nutrients from the upland (e.g., septic systems; farms; golf courses). Over the past century, non-native *P. australis* has been aggressively invading coastal marshes of North America (see reviews in Chambers, Meyerson, & Saltonstall, 1999; Meyerson, Saltonstall, Windham, Kiviat, & Findlay, 2000), and shoreline development at the upland border of salt marshes in New England is correlated with increasing cover of invasive *P. australis* (Bertness, Ewanchuk, & Silliman 2002; Silliman & Bertness, 2004). Therefore, if the proximity of the plant species to the nitrogen source is important and influences the stable nitrogen isotope ratio, the δ^{15}N of border salt marsh plants might more closely reflect the local upland anthropogenic nitrogen sources rather than an integrated watershed-sea signal.

The low marsh dominant plant, *S. alterniflora* is tidally flushed twice a day in New England and may best reflect an integrated isotope signal that combines a number of sources including land-derived groundwater intrusion and mixing from the sea. In urbanized areas the estuarine waters may have a nitrogen isotope signal that is also influenced by sewage inputs. Those sites closest to sewage outfalls in the estuary might have a more elevated stable nitrogen isotope signal than the sites downbay from the sewage outfall (Pruell, Taplin, Lake, & Jayaraman, 2006). The high marsh dominant plant, *Spartina patens* (Ait.) Muhl also experiences increased soil saturation of sea water with the rise of the tide, but only occasional flooding in New England. In addition, *S. patens*, in contrast to *S. alterniflora*, is mycorrhizal and the fungal symbionts in the roots of *S. patens* are reported to indirectly facilitate nitrogen fixation in the rhizosphere (Burke, 2001, Burke, Hamerlynck, & Hahn, 2002). This new source of fixed atmospheric nitrogen to the roots would likely lower the stable nitrogen isotope ratio of *S. patens* compared to *S. alterniflora*. Nitrogen fixation by microalgal epiphytes on dead stems of *S. alterniflora* contributes to the downward shift of the stable nitrogen isotope ratios of senescent plants compared to live ones (Currin, Newell, & Paerl, 1995).

In the present study, using new and published data, we examine the relationships of anthropogenic stressors (i.e., human wastewater nitrogen; land use) with the stable nitrogen isotope ratios of two green seaweeds, *Ulva lactuca* (L.) and *Enteromorpha intestinalis* (L.) and four vascular plants, *I. frutescens*, *P. australis*, *S. patens*, and *S. alterniflora* collected from sites in the urbanized northeast USA. With increasing residential land development, we expect increasing wastewater nitrogen inputs into the marshes and elevated δ^{15}N values in the plants. For the tidally flushed and rooted emergent *Spartina* plants, we expect a downward shift in the stable nitrogen isotope ratios of *S. patens* compared with *S. alterniflora*, which we propose reflects the contribution of fixed nitrogen by the *S. patens* mycorrhizal roots. In addition, the relationships of percent agricultural lands with δ^{15}N values of the plant species is examined. A downward shift in δ^{15}N values in border plant species near coastal farms due to the contribution of commercial, inorganic fertilizers is expected. In high residential areas, we examine whether the δ^{15}N of distinct stands of non-native *P. australis* adjacent to upland woody and shrub buffers have lower δ^{15}N values than plants without vegetated buffers supporting the hypothesis that the vegetated buffer acts as a sink for upland sources of wastewater nitrogen (McClelland & Valiela, 1998; McClelland et al., 1997).

2 Materials and Methods

The location of the Narragansett Bay, RI and Cape Cod, MA salt marsh sites in New England are shown in Figure 1. The watershed land use and estimates of wastewater nitrogen inputs for the 10 Narragansett Bay salt marsh sites (Wigand et al., 2001, Wigand, McKinney, Chintala, Charpentier, & Thursby, 2003) and the six Cape Cod salt marsh sites have been previously reported and are summarized in Table I (Cole et al., 2004; McClelland & Valiela, 1998; McClelland et al., 1997; Valiela et al., 1997). The nitrogen loads for the salt marsh sites are estimated using a nitrogen-loading model (NLM) developed and verified for Cape Cod, MA by Valiela et al. (1997) and Valiela, Geist, McClelland, and Tomasky (2000). The NLM estimates coastal nitrogen loads from atmospheric deposition, fertilizer, and wastewater (e.g., via septic systems, using values for per capita contributions of nitrogen) in watersheds by multiplying the surfaces of various land use types (e.g., natural vegetation, agricultural land, turf, residential land and impervious surfaces) by an appropriate coefficient and subsequently correcting the loads for nitrogen losses in various compartments (e.g., vegetation and soils, vadose zone, aquifer). Percent wastewater nitrogen loads and land use cover for the RI and MA sites used in this study have been previously reported and also are summarized in Table I.

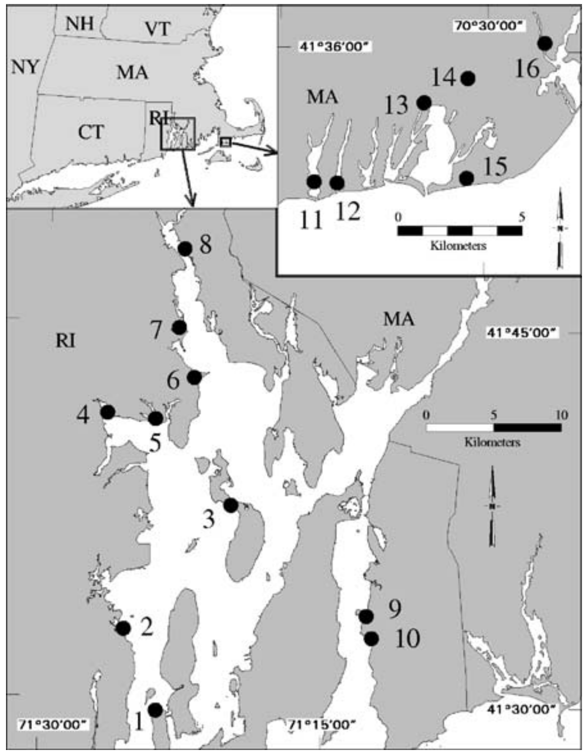

Figure 1 The location of the salt marsh study sites in Narragansett Bay, RI and Cape Cod, MA in New England. The identifications of the numbered sites are found in Table I.

We examined the relationships of the stable nitrogen isotope ratios of various coastal plant species with land use and percent wastewater nitrogen. The mean stable nitrogen isotope ratios for the MA plant species (i.e., *U. lactuca*, *E. intestinalis*, *S. alterniflora*) were previously determined (Cole et al., 2004; McClelland et al., 1997) as was the stable nitrogen isotope ratios for the *S. alterniflora* at the RI sites (Wigand et al., 2001, 2003). In MA the S. alterniflora samples (n=3 plants from each of 10–15 locations within a site) were collected at regular intervals by hand along the salt marsh bank at the water's edge and macroalgae samples (n=3 each from 10–15 locations within a site) were collected at regular intervals along the shoreline of each estuary (McClelland et al., 1997; Cole et al., 2004). Primary producers sampled at the MA sites were collected during the growing season, oven-dried to a constant weight, and homogenized into a composite sample for each species at each site for each date sampled. For the *S. alterniflora, S. patens,* and *I. frutescens* collected at the RI sites, the stable nitrogen isotope ratios for at least three distinct

Table I Percent wastewater are nitrogen loads and land use cover for the RI and MA sites used in this study. See Figure 1 for a map of the location of the salt marsh sites

State	Map identification #	Site	Percent (%) wastewater *N*	Percent (%) residential	Percent (%) agriculture
RI[a]	1	Fox Hill Pond	0.7	0.3	56.6
	2	Bissel Cove	68.5	22.1	19.9
	3	Jenny Creek	20.3	4.0	1.9
	4	Apponaug Cove	83.3	43.3	12.3
	5	Brush Neck Cove	86.9	61.8	9.6
	6	Old Mill Creek	84.9	44.5	11.0
	7	Passeonquis Cove	87.1	65.4	9.9
	8	Watchemoket Cove	82.0	56.0	15.0
	9	Fogland Marsh	20.8	14.9	72.6
	10	Mary Donovan Marsh	25.7	10.0	58.5
MA[b]	11	Great Pond	66.0	58.0	14.9
	12	Great Pond	54.0	67.0	25.0
	13	Childs River	65.0	69.0	12.4
	14	Quashnet River	30.0	18.0	26.6
	15	Sage Lot Pond	0.05	6.0	64.9
	16	Mashpee River	44.0	24.0	8.8

[a] RI wastewater and land use based on Wigand et al. 2001, 2003

[b] MA wastewater and land use based on McClelland et al. 1997 and Cole et al. 2004

plant stands (n=10 plants per stand) on each date were sampled. *U. lactuca* collected in RI was sampled from at least three distinct locations at the salt marsh edge at each site. The number of dates that the stable nitrogen isotope ratios were measured for each species at the MA and RI sites varied, and ranged from one to five (Cole et al., 2004; McClelland et al., 2004; Wigand et al. 2001). Mean stable nitrogen isotope ratios for each primary producer at each site are reported and used in the regression analyses in the study (Table II).

The *P. australis* was sampled during the 2000 growing season at 10 Narragansett Bay, RI sites. Only one native population of *P. australis* has been reported in the state of RI (on Block Island: Lambert, 2005), and examination of plant morphological characteristics (i.e., ligules and glumes detailed in Saltonstall, Peterson, & Soreng, 2004) indicated that all of the *P. australis* sampled in this study were non-native. All distinct non-native *P. australis* stands (i.e., those plant patches with distinct borders that separated the *Phragmites*-dominated community from other more diverse plant communities) at each site were sampled. We observed with the naked eye or binoculars the land use and cover adjacent to the upland border of the salt marsh and approximately 50 m landward to each distinct *P. australis* stand. Only green leaves, generally, the third youngest leaf on 10 semi-randomly selected plants in the approximate center of the stand were collected, dried, and ground. Subsequently, the stable nitrogen isotope ratio for the homogenized sample for each distinct *P. australis* stand was determined as described below.

The stable nitrogen isotope ratios of the plants in previous studies were measured by isotope ratio mass spectrometry using standard methods (Cole et al., 2004; McClelland et al., 1997; Wigand et al., 2001). For the *P. australis* samples collected and processed in the present study, nitrogen isotope composition was determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) employing a Carlo–Erba NA 1500 Series II Elemental Analyzer interfaced to a Micromass Optima Mass Spectrometer. The nitrogen isotope ratio of the tissue is expressed as a part per thousand (per mil) difference from the composition of a recognized reference material, N_2 in air (Mariotti, 1983). All samples were analyzed in duplicate with a typical difference of about 0.1‰. Sample material reanalyzed periodically over a several month period exhibited a precision of ±0.30‰, calculated as a single standard deviation of all replicate values. This latter estimate of precision is

Table II The mean stable nitrogen isotope ratios for plant species collected from RI and MA salt marshes. The published sources for the stable nitrogen isotope data are indicated

State	Map identification #[c]	Site	$\delta^{15}N$ (‰)				
			S. alterniflora	*S. patens*	*I. frutescens*	*E. intestinalis*	*U. lactuca*
RI[a]	1	Fox Hill Pond	6.8	2.6	0.2	ND	7.8
	2	Bissel Cove	7.9	6.2	2.2	ND	ND
	3	Jenny Creek	7.2	5.7	3.1	ND	ND
	4	Apponaug Cove	9.5	8.2	7.3	ND	ND
	5	Brush Neck Cove	9.3	6.8	5.0	ND	12.1
	6	Old Mill Creek	11.0	7.7	4.4	ND	ND
	7	Passeonquis Cove	9.4	6.1	9.1	ND	11.5
	8	Watchemoket Cove	8.4	6.6	6.8	ND	9.6
	9	Fogland Marsh	7.9	5.3	-1.2	ND	ND
	10	Mary Donovan Marsh	7.8	5.2	0.6	ND	ND
MA[b]	11	Great Pond	7.7	ND	ND	9.9	8.3
	12	Great Pond	8.1	ND	ND	7.3	8.1
	13	Childs River	7.6	ND	ND	8.4	ND
	14	Quashnet River	6.0	ND	ND	6.4	ND
	15	Sage Lot Pond	4.4	ND	ND	4.9	ND
	16	Mashpee River	6.9	ND	ND	7.5	6.9

ND No data available

[a] RI plant stable nitrogen isotope ratios based on Wigand et al. 2001 and C. Wigand, unpublished data

[b] MA stable nitrogen isotope ratios based on McClelland et al. 1997 and Cole et al. 2004

[c] See Figure 1 for the location of the marsh sites.

appropriate for $\delta^{15}N$ values measured as part of this study.

In the present study we use a landscape-level approach to examine for relationships between different marsh plant stable nitrogen isotope ratios and anthropogenic stressors. We developed our hypotheses for relating the $\delta^{15}N$ of different plant species on the New England marsh landscape with watershed anthropogenic stressors based on previously published landscape, space-for-time substitution studies (McClelland & Valiela, 1998; McClelland et al., 1997; Valiela et al., 1997; Wigand et al., 2003), and manipulative experiments that provided some mechanistic understanding of the effect of nutrient enrichment on the plant community structure of New England marshes (Emery, Ewanshuk, Bertness, 2001; Levine, Brewer, & Bertness, 1998; Minchinton & Bertness, 2003). Regression analyses were used to examine for the relationships of percent wastewater nitrogen and land use with the mean stable nitrogen isotope ratios for the six different plant species. Pairwise *t*-tests were used to examine for differences in $\delta^{15}N$ among selected plant species sampled from the same marsh sites. In high residential areas (>40%) a *t*-test was used to test whether there was a significant difference in $\delta^{15}N$ of the *P. australis* stands with and without vegetated (i.e., woody or shrub) buffers along the upland border. In this analysis, we excluded salt marshes with adjacent watersheds of low residential development (<25%) and one *Phragmites* stand without a buffer that was adjacent to an industrial sewage transfer station observed to leak (C. Wigand, personal observations). The probability for significance is reported at $P<0.05$ for all statistical analyses.

3 Results

Both *Spartina* species showed significant relationships between estimates of wastewater nitrogen and the mean stable nitrogen isotope ratios (Figure 2). Wastewater nitrogen could explain about 62% and 70% of the variability in the stable nitrogen isotope ratios of *S. alterniflora* and *S. patens*, respectively. Slopes of the linear relationships for both species

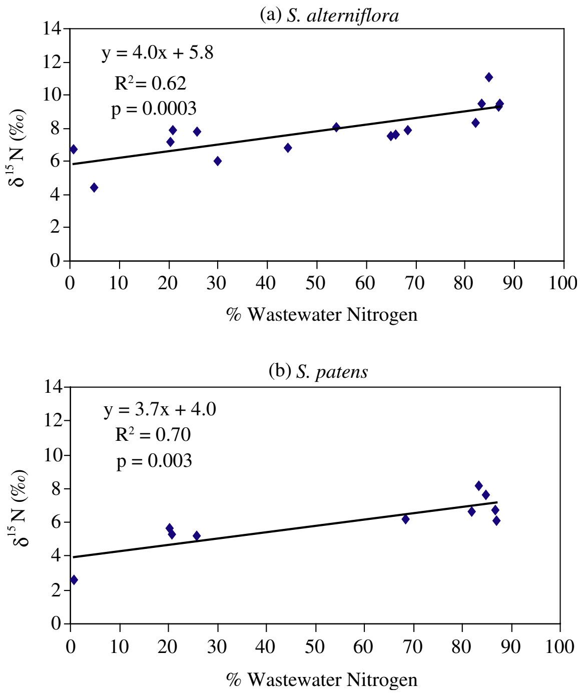

Figure 2 The regression relationships of percent wastewater nitrogen with the stable nitrogen isotope ratios of the *Spartina* species, **a***S. alterniflora*, **b***S. patens*.

were similar, but the y-intercept of *S. patens*, which is mycorrhizal, was about 2‰ lower than *S. alterniflora* (Figure 2). There was a significant ($P<0.0001$) downward shift in the $\delta^{15}N$ of *S. patens* (6.0± 0.48‰) compared with *S. alterniflora* (8.5±0.41‰) (Table III).

Significant relationships were also found between stable nitrogen isotope ratios and percent residential land use for the *Spartina* species, but these relationships were not as strong as those with wastewater nitrogen (Table IV). The significant relationships with percent residential land development are expected since the wastewater nitrogen estimates are based on a land-based model (Valiela et al., 1997), and the percent residential development is the most dominant type of land use in this urbanized region. The percent residential land development could explain 33% and 45% of the variability in the stable nitrogen isotope ratios for *S. alterniflora* and *S. patens*, respectively. For both *Spartina* species the inverse relationships of $\delta^{15}N$ and percent agricultural development were weak, and only significant for *S. patens* (Table IV). In contrast, the inverse relationships of $\delta^{15}N$ and percent agricultural land use were significant for the marsh upland-border species, *I. frutescens* and *P. australis* (Table IV). The percent agricultural development could explain 64% and 49% of the variability in the stable nitrogen isotope ratios of *I. frutescens* and *P. australis*, respectively (Table IV).

For sites with >50% agricultural lands in the watershed, the $\delta^{15}N$ of the *I. frustescens* was <1‰, but for the other marsh plant species sampled at these sites the $\delta^{15}N$ ranged from 2.6‰–7.9‰ (Tables I, II). In pairwise *t*-tests, the $\delta^{15}N$ of *I. frutescens* was significantly lower than *S. alterniflora*, *S. patens*, and *P. australis* (Table III).

I. frutescens and *P. australis* showed significant relationships between $\delta^{15}N$ and wastewater nitrogen (Figure 3). For *I. frutescens*, wastewater nitrogen could explain about 68‰ of the variability in $\delta^{15}N$. *Iva frutescens* showed the only negative *y*-intercept in this study (Figure 3) which may be attributed to the low

Table III Pairwise *t*-test of the stable nitrogen isotope ratio ($\delta^{15}N$) of marsh plants at RI and MA sites

Species comparison of ($\delta^{15}N$) (mean ‰ ± SE)		# sites (*n*)	*P* value*	Site location
S. alterniflora 8.5±0.41	*S. patens* 6.0± 0.48	10	<0.0001	RI
I. frutescens 3.7± 1.07	*S. alterniflora* 8.5±0.41	10	<0.001	RI
I. frutescens 3.7±1.07	*S. patens* 6.0±0.48	10	<0.05	RI
I. frutescens 3.8±1.19	*P. australis* 8.3 ±0.86	9	<0.005	RI
S. alterniflora 8.7±0.42	*P. australis* 8.3 ±0.86	9	NS	RI
S. patens 6.1±0.54	*P. australis* 8.3 ±0.86	9	<0.01	RI
S. alterniflora 6.8±0.56	*E. intestinalis* 7.4±0.69	6	NS	MA
S. alterniflora 8.1±0.41	*U. lactuca* 9.2±0.74	7	<0.05	RI & MA

NS Not significant

**P* value reported significant at $P<0.05$

Table IV Regression relationships of the percent residential and agricultural land development with the stable nitrogen isotope ratios of six marsh plant species in New England

		Percent residential land		Percent agricultural land	
Plant species	*N*	R^2	*P*	R^2	*P*
S. alterniflora	16	0.33	0.02	0.22	0.06
S. patens	10	0.45	0.03	0.43	0.04
I. frutescens	10	0.72	0.002	0.64	0.006
P. australis	9	0.48	0.04	0.49	0.04
E. intestinalis	6	0.57	0.08	0.65	0.052
U. lactuca	7	0.35	0.17	0.16	0.37

δ^{15}N of plant material collected at the RI sites with active coastal farms nearby (Fogland Marsh, Mary Donovan Marsh, and Fox Hill Pond; Tables I, II). For *P. australis* only 53% of the variability in stable nitrogen isotope ratios could be explained by the percent wastewater nitrogen (see Figure 3). The distinct stands of *P. australis* within sites had wide ranges of δ^{15}N values (Table V). At one of the most impacted RI marshes (i.e., Apponaug Cove), with high residential development and wastewater nitrogen loads, there were seven stands with δ^{15}N values ranging from −1‰ to 15‰. At the Apponaug marsh, we generally measured higher δ^{15}N in stands adjacent to homes with no vegetated buffers, intermediate δ^{15}N values in stands adjacent to homes with woody or shrub buffers, and a single low value (−0.9‰) in a stand adjacent to an apparently over-fertilized lawn. In contrast, three sparse and somewhat stunted *P. australis* stands located on the tidally flushed Sakonnet River (one stand at Fogland marsh and two at Mary Donavan marsh) where there was low residential development and no adjacent farms averaged a

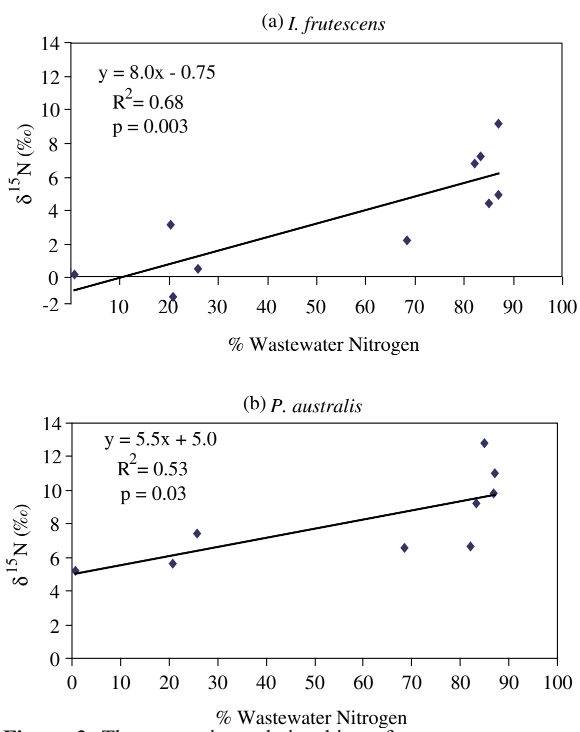

Figure 3 The regression relationships of percent wastewater nitrogen with the stable nitrogen isotope ratios of **a** *I. frutescens* and **b** *P. australis*.

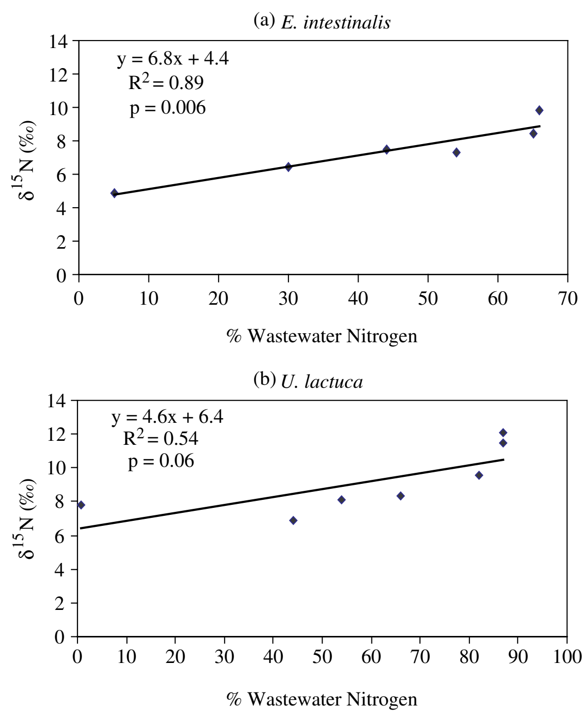

Figure 4 The regression relationships of percent wastewater nitrogen with the stable nitrogen isotope ratios of **a** *E. intestinalis* and **b** *U. lactuca*.

Table V The mean stable nitrogen isotope ratios, ranges, and standard error of the means for stands of *P. australis* collected from salt marsh sites in Narraganset Bay, RI

Map identification #	Site	# Phragmites stands	δ^{15}N (‰)	Minimum	Maximum	SE
1	Fox Hill Pond	2	5.2	5.0	5.4	0.2
2	Bissel Cove	3	6.6	3.5	8.4	1.6
3	Jenny Creek	0	NA	NA	NA	NA
4	Apponaug Cove	7	9.2	-0.9	14.5	1.9
5	Brush Neck Cove	5	9.8	7.0	12.9	1.3
6	Old Mill Creek	4	12.8	8.9	16.4	1.9
7	Passeonquis Cove	2	11.0	9.5	12.6	1.6
8	Watchemoket Cove	3	6.7	4.6	8.1	1.1
9	Fogland Marsh	3	5.7	4.2	6.7	0.8
10	Mary Donovan Marsh	14	7.4	3.9	10.3	0.5

NA Not applicable

δ^{15}N of 4.1±0.09‰. Comparing among sites, the δ^{15}N of *P. australis* was not significantly different from the δ^{15}N of *S. alterniflora*, but was significantly greater than *S. patens* (Table III).

Additionally, the woody or shrub buffers adjacent to the *P. australis* stands in areas with developed watersheds caused a significantly lower δ^{15}N (mean=7.1±0.88‰; n=11 stands) compared to *P. australis* stands where there was no vegetated buffer at the upland border (mean=12.3±0.90‰; n=11) (Figure 5, $P<0.001$). These results are likely conservative because in the group without woody or shrub buffers, we included sites with lawns adjacent to the *P. australis* stands. While human wastewater nitrogen would likely increase the δ^{15}N of the *P. australis*, the commercial, inorganic fertilizers often used on lawns would have the opposite effect (McClelland et al., 1997). Nevertheless, we did measure significantly lower δ^{15}N in *P. australis* stands with the upland woody and shrub buffers. These vegetated buffers were 3–50 m in width.

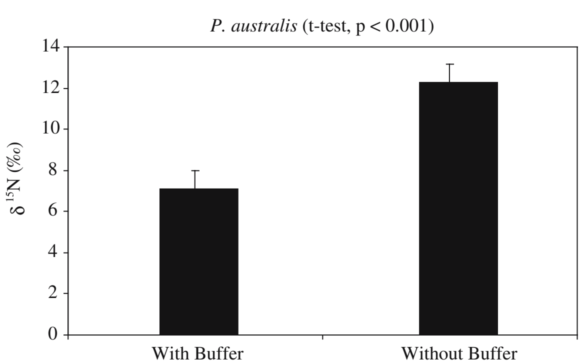

Figure 5 Comparison of the stable nitrogen isotope ratios of non-native *Phragmites* stands with and without upland vegetated buffers in Narragansett Bay, RI.

The macroalga, *E. intestinalis*, showed a significant relationship of δ^{15}N with wastewater nitrogen (Figure 4). There were trends of association, but no significant relationships of the δ^{15}N of *E. intestinalis* with percent residential or agricultural land use (Table IV). In addition, there were no significant relationships among the δ^{15}N of *U. lactuca* and wastewater nitrogen, percent agricultural or residential land use (Figure 4, Table IV). Albeit not significant, there was a trend of increasing δ^{15}N in *U. lactuca* with increasing wastewater nitrogen (R^2=0.54; P=0.06). While the δ^{15}N of *E. intestinalis* is similar among sites to *S. alterniflora*, the δ^{15}N of *U. lactuca* was significantly greater than *S. alterniflora* (Table III).

4 Discussion

Some coastal well-flushed marsh plants (e.g., *S. alterniflora*) are watershed-sea integrators of anthropogenic nitrogen (McClelland et al., 1997; Cole et al., 2004), while other plants (e.g., non-native *P. australis*) may be good indicators of more local nitrogen disturbances along the upland edge (Bertness et al., 2002; Silliman & Bertness, 2004). The land immediately adjacent to the *I. frutescens* and *P. australis* stands, for instance a leaky septic system, farm, lawn, or vegetated buffer, may influence the δ^{15}N of the plants. High residential development near the upland edge could contribute local sources of wastewater nitrogen (δ^{15}N ranging from +10‰ to +20‰) to the plants, for example, from a leaky septic system which would increase the δ^{15}N of the *P. australis*. On the other hand, commercial fertilizers (δ^{15}N ranging from −3‰ to +3‰) might decrease the δ^{15}N values of the plants, for example, if a stand was adjacent to an over-fertilized lawn or farm (for δ^{15}N ranges see McClelland et al., 1997). Or, in sites with high residential development, the presence of a woody or shrub buffer adjacent to *P. australis* may result in a relatively lower δ^{15}N than in stands without vegetated buffers (see Figure 5).

We measured <1‰ δ^{15}N for only some *I. frutescens* and *P. australis* stands, apparently driven by local, upland inputs of commercial, inorganic fertilizers to the marsh border. The δ^{15}N of *I. frutescens* was significantly related with agricultural land use with low δ^{15}N values (<1‰) at sites with high coastal agricultural lands (>50%). The fertilizer use on the farms was apparently driving the δ^{15}N of *Iva* downwards. The other marsh plant species at the sites with >50% agricultural lands had higher δ^{15}N values (averages ranging from 2.6‰–7.9‰), possibly driven up by the exchange of dissolved nitrogen from the sea or residential wastewater nitrogen inputs.

Unlike the native *I. frutescens* stands which were only found in the high marsh and at the upland edge, the coverage of the non-native *P. australis* stands was more variable. *P. australis* stands were usually found in the high marsh, but sometimes extended from the upland border to the sea. The *P. australis* stands were also present and sometimes widespread in residential and agricultural watersheds. Silliman and Bertness (2004) show that with increasing shoreline development in the upland border of New England marshes, the nitrogen availability for growth and the percent of the border dominated by *P. australis* increases. Among *P. australis* stands in our study, there was high within site variability in δ^{15}N (Table V), evidently caused by the presence of agricultural land, residential land, or vegetated buffer at the upland border.

In a review of the estimated percent removal of various pollutants by vegetated buffers in coastal areas, Desbonnet et al., (1995) reported that over 70% of the nonpoint source nitrogen inputs were removed by vegetated buffers of 25 m or greater and 50% of the nitrogen was removed by buffers of only 3.5 m widths. It is likely that the woody and shrub buffers (3–50 m) in our study act as sinks for diffuse sources of anthropogenic nitrogen derived from the watershed (McClelland & Valiela, 1998; McClelland et al., 1997). In areas with high residential development it is likely that vegetated buffers cause relatively lower δ^{15}N in *P. australis* stands because wastewater nitrogen is sequestered by vegetation in the buffer. In manipulative experiments, Minchinton and Bertness (2003) demonstrate that the removal of border vegetation (i.e., marsh grasses and rushes) adjacent to *P. australis* promotes the spread of the plant, apparently because of the removal of competitors for nutrients.

In this marsh study, the stable nitrogen isotope ratio of *U. lactuca* did not have a clear relationship with the watershed anthropogenic stressors. Although both *U. lactuca* and *E. intestinalis* are macroalgae commonly associated with salt marsh systems in urbanized localities, *E. intestinalis* is more often attached and erect allowing it to be bathed by nutrient-laden water. *U. lactuca* is often lying flat on the marsh landscape where microbial cycling of organic nitrogen could influence the δ^{15}N signal and also, *U. lactuca* may have been brought in by the tides from the main stem of the estuary. *U. lactuca* from the mainstem of the Narragansett Bay estuary may reflect a sewage signal as reported by Pruell et al. (2006).

In addition, Pruell et al. (2006) found that the δ^{15}N of *S. alterniflora* was not a good biological indicator of the sewage signal in the main stem of Narragansett Bay as one moves from the more urbanized head of the bay to the less populated mouth. The δ^{15}N of *S. alterniflora* better reflects the integrated signal of both estuarine (i.e., natural sea and sewage inputs) and diffuse watershed sources as shown in this study and previous ones in New England (McClelland et al., 1997; McClelland & Valiela, 1998; Wigand et al., 2001, 2003; Cole et al., 2004).

When coastal wetland managers are choosing indicator plants for linking with anthropogenic nitrogen sources, some important parameters to consider include: the proximity of the plant to the nitrogen source, the presence of vegetated buffers, hydrologic flowpath, flooding regime, and plant physiology. Some plant species such as *S. patens* and *S. alterniflora* have different δ^{15}N values, apparently because of the assimilation of fixed nitrogen in the mycorrhizal roots of *S. patens*. However, the two *Spartina* species had similar slopes when regressing plant δ^{15}N with wastewater nitrogen suggesting that with increasing wastewater nitrogen loads the two species are assimilating increasing amounts of wastewater nitrogen at similar rates. In urbanized New England salt marshes, the stable nitrogen isotope ratios of *I. frustescens* and *P. australis* stands appear to be good indicators of local, anthropogenic nitrogen inputs near the upland border, while the δ^{15}N of *Spartina* species on the tidally flushed marsh landscape relates with the integrated, watershed-sea nitrogen inputs.

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